Norwegian Institute for Water Research

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Quality Assurance Project Plan (QAPP)

- for Shipboard testing of the PureBallast ballast water treatment system of Alfa Laval

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Abbreviations and acronyms

APHA – American Public Health Association
Wallenius AOT Module – Wallenius Advanced Oxidation Technology Module
CFDA-AM - 5-carboxyfluorescein diacetate acetoxymethyl ester
DNV – Det Norske Veritas
DO – dissolved oxygen
DOC – dissolved organic carbon
DQIs – data quality indicators
FNU – Formazine Nephelometric Units
GF/F – glass fiber filter grade F
GLP – Good laboratory Practice
IMO – International Maritime Organization
ISO – International Organisation for Standardization
n – number of measurements; in calculating the standard deviation
NDIR - Nondispersive Infrared
NIVA – Norwegian Institute for Water Research
NS-EN ISO – Norwegian, European and International Standard
OECD – Organisation for economic Co-operation and Development
PAR – photosynthetic active radiation
POC – particulate organic carbon
PSU – Practical Salinity Unit (= ‰)
QAPP – quality assurance project plan
QA/QC – quality assurance/quality control
QMP – quality management plan
Std – standard deviation
TCBS - MacConkey and thiosulphate citrate bile salt agar
TSS – total suspended solids
TCW1 - Tank for control water
TCW2 - Tank for control water
TTW1 - Tank for treated water
TTW2 - Tank for treated water
X_i - individual analytical result; in calculating the standard deviation
X – the arithmetic mean of individual analytical results; in calculating the standard deviation

1. Project description, objectives and organisation

1.1 Address of QAPP

This QAPP describes the implementation of quality assurance and quality control activities during the evaluation of the Alfa Laval ballast water management system, PureBallast, according to the requirements for shipboard testing stated in the IMO Guidelines for Approval of Ballast Water Management Systems (G8) (MEPC 53/24/Add.1, Annex 3, Part 2, 2005). The requirements involve all parts and processes of the test as listed in:

Annex - Guidelines for approval of ballast water management systems (G8)

- Part 2 – Test and performance specifications for approval of ballast water management systems
- 2.1 Quality assurance and quality control procedures (page 15)
- 2.2 Shipboard tests (pages 15-17)
- 2.4 Reporting of Test Results (page 22)
- Part 4 – Sample analysis methods for the determination of biological constituents in ballast water (page 24-26)

The QAPP is a mean to reveal any problems before start-up and during execution of the project at as early stage as possible to minimize any potential procedural, technical and scientific inadequacies and time- and economic losses. This QAPP will be used for biological evaluation in accordance with the IMO guidelines.

1.2 Processes to be evaluated

The QAPP will cover all parts of the project, both preparations, execution of tests and reporting of results;

1.2.1 Preparations

- Shipboard preparation of the PureBallast water management system according to its operating manual

1.2.2 Execution of tests

- Running the PureBallast water management system according to its operating manual
- Monitoring the operational performance of the ballast water management system itself
- Sampling for chemical and biological samples of uptake/source/influent water
- Sampling for biological samples of discharge control water
- Sampling for biological samples of discharge treated water
- Performing chemical analyses
- Performing biological analyses

1.2.3 Reporting of results

- Statistical evaluation of the results with regards to the D2 regulation determined by the IMO Convention (IMO, 2004)
- Reporting of the results to the Administration according to the requirements in the IMO Guideline as specified in section 2.4 of Annex 3 (MEPC 53/24/Add.1, 2005)

1.3 Description of ship and technology set-up

1.3.1 Ship and shipboard installation

The test will be conducted at Wallenius Marines car carrier M/V Aida (see Figure 1).

Figure 1 Wallenius Car Carrier M/V Aida.

Configuration of the test tanks with pipes, valves and devices is shown in Figure 2 and Figure 3. The tanks will have the following labelling (Table 1):

Table 1 Labelling of ballast tanks used for testing.

Description
Tank for control water (TCW1)
Tank for control water (TCW2)
Tank for treated water (TTW1)
Tank for treated water (TTW2)

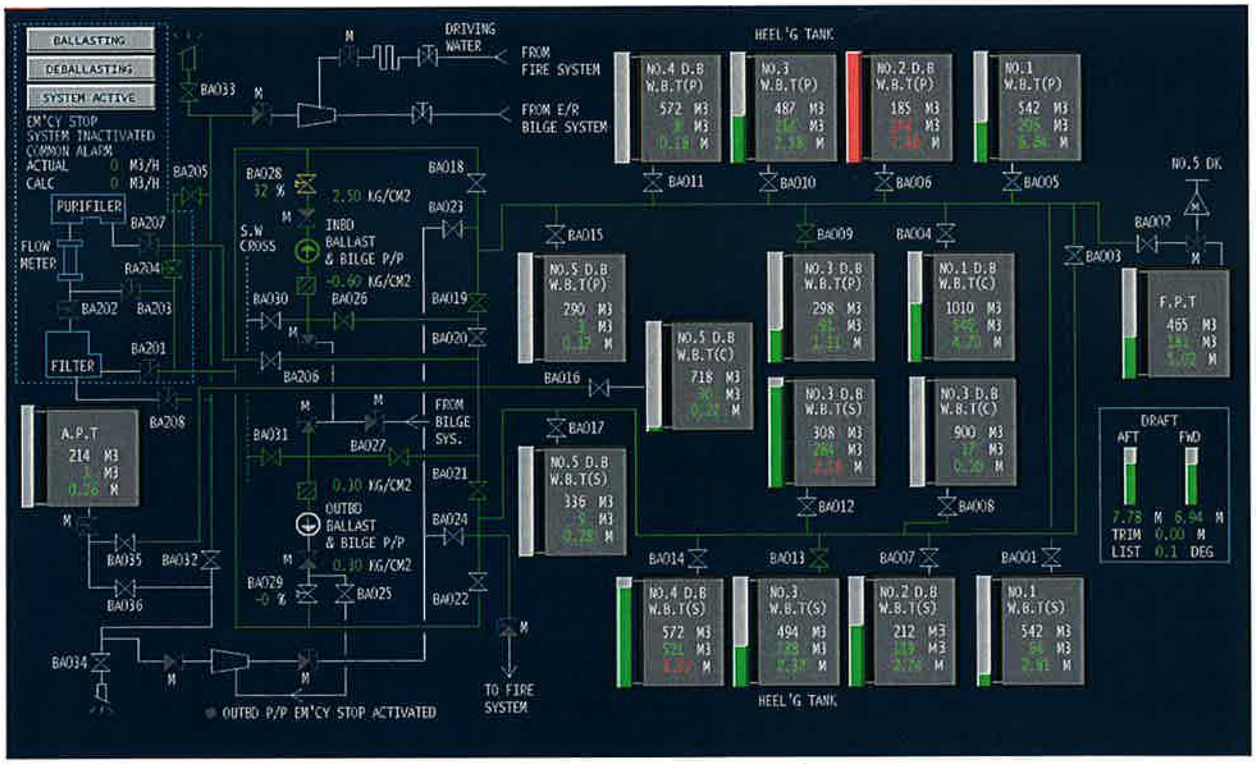


Figure 2 Configuration of the test tanks with pipes, valves and devices.

8. SUMMARY OF TANK CAPACITY

1) WATER BALLAST TANKS

(S.G.= 1.025)

COMPARTMENT	LOCATION (FR.NO.)	CAPACITIES		CENT OF GRAVITY		MAX. F.S.M. (M**4)
		VOLUME 100% (M**3)	WEIGHT 100% (TONS)	L.C.G (M)	V.C.G (M)	
F.P.TK	227-243	465.2	476.8	92.15	6.66	181
NO.1 W.B.TK(P)	186-221	542.1	555.6	64.64	10.18	705
NO.1 W.B.TK(S)	186-221	542.1	555.6	64.64	10.18	705
NO.1 DB.W.B.TK(C)	186-216	1010.2	1035.5	68.09	4.33	1691
NO.2 DB.W.B.TK(P)	162-186	185.1	189.7	44.39	2.84	183
NO.2 DB.W.B.TK(S)	162-186	212.4	217.7	45.48	3.43	173
NO.3 DB.W.B.TK(C)	138-186	899.8	922.3	31.33	1.58	3096
NO.3 DB.W.B.TK(P)	126-159	298.0	305.5	16.72	1.96	747
NO.3 DB.W.B.TK(S)	126-162	308.4	316.1	17.30	1.97	777
NO.3 W.B.TK(P)	126-183	487.3	499.5	30.55	11.23	258
NO.3 W.B.TK(S)	132-186	494.1	506.5	35.13	11.30	318
NO.4 DB.W.B.TK(P)	90-126	571.7	586.0	-8.67	1.83	1951
NO.4 DB.W.B.TK(S)	90-126	571.7	586.0	-8.67	1.83	1951
NO.4 DB.FIXED B.TK(C) *	90-138	1239.5	2850.8	-3.55	1.55	4596
NO.5 DB.W.B.TK(C)	52- 90	717.0	734.9	-33.41	1.56	4225
NO.5 DB.W.B.TK(P)	54- 87	290.2	297.5	-34.94	1.94	793
NO.5 DB.W.B.TK(S)	54- 90	336.0	344.4	-33.44	1.92	968
A.P.TK(C)	-5- 12	213.8	219.2	-91.91	9.71	1487
TOTAL		9384.6	11198.8			

* NO.4 DB.FIXED B.TK(C) S.G.= 2.300

Figure 3 Overview of Ballast Water Tanks for M/V Aida.

1.3.2 The PureBallast ballast water management system

1.3.2.1 Process description

A process description with the chemical features and the discharge characteristics of the Wallenius AOT Process is found in "Appendix G – Process description".

1.3.2.2 System and component description

The PureBallast system of Alfa Laval is a complete ballast water treatment system composing of three main component ballast water treatment system; 1) a 50 µm filter for removal of larger particles and organisms, 2) the Wallenius AOT (Advanced Oxidation Technology) Module, a patented treatment system using the synergetic effects of *in situ* produced free radicals and direct photo-radiation to inactivate microbes and 3) control and auxiliary equipment including sampling points to control water flows and measure different alarm levels during operation as well as to take samples during operation and testing.

The system and its components are further described in "Appendix H – Description and the PureBallast system and its components".

1.3.2.3 Physical properties of the PureBallast test equipment

The test equipment is a complete PureBallast system with a flow rate of max 1000 m^3/h. The system has one pre-filter and four Wallenius AOT units with capacity of 250 m^3/h each and a CIP-unit for cleaning the AOT units..

Figure 4 The PureBallast testsystem. AOT units to the left and pre-filter and CIP-unit to the right.

1.4 Organization of the project

The project is a joint cooperation between AlfaWall AB as the vendor, Norwegian Institute for Water Research (NIVA) as testing organization, and Det Norske Veritas (DNV) as the verification organization (on behalf of the Noweigan administration).

1.4.1 Presentation of Norwegian Institute for Water Research (NIVA) - testing site owner and testing organization

NIVA is Norway's leading multidisciplinary research institute in the field of use and protection of water. NIVA has more than 80 scientists in the fields of chemistry, biology, limnology, geology, hydrology, environmental technology, environmental toxicology, oceanography, geography, resource management and economy. NIVA has managed several national and international projects related to water and wastewater treatment, ecotoxicology and risk assessment, including testing and verification of ballast water treatment technologies in laboratory and pilot scale. These projects have been funded by The European Community, The Research Council of Norway and private enterprises.

NIVA has well-equipped laboratories for accredited chemical and biological analysis, and ecotoxicological assays, located in Oslo. NIVA is also the owner of Marine Research Station Solbergstrand, an experimental laboratory for large scale experiments and tests, situated at Oslo Fjord.

1.4.2 Quality management plan (QMP) of NIVA

The following documents describe NIVA's quality system:

- Quality manual for accredited laboratory analyses and tests. In 1993 NIVA's Chemical Laboratory, which provides the widest scope of analyses of environmental and water samples in Norway, was accredited according to Norwegian Standard NS-EN ISO/IEC 17025 (Norwegian Accreditation, 1993). NIVA performs annually 150.000 analyses of water quality including nutrients, metals, unspecified organic material, organic micropollutants and test of marine soft-bottom fauna. The accreditation gives the customer a warranty that NIVA´s laboratories use analytical procedures according to internationally acknowledged quality systems (NS-EN ISO/IEC 17025).

- Quality manual for ecological testing based on OECD Principles of Good Laboratory Practice. NIVA now performs ecotoxicity testing according to international standards and is registered as a GLP laboratory in Norway (reg. no GLP 007) and is regularly inspected by the Norwegian Metrology and Accreditation Services. Our ecotoxicological laboratory performs standardised tests (e.g. in accordance with OECD guidelines or ISO standards) as well as special services concerning toxicity, biodegradability and bioaccumulation of chemical substances, products and complex mixtures, e.g. industrial effluents and environmental samples.

- Quality manual for internal control (health, environment and safety). The quality system for internal control (health, environment and safety) is described in; 1) a primary document comprising the principles that are implemented for internal control in health, environment and safety issues, 2) an operative part comprising operating procedures and 3) a documenting part for health, environment and safety related documentation.

NIVA is at present in a process with the aim of obtaining an ISO9000 accreditation of the management of projects.

In some cases, local laboratories, such as Intertek laboratories, Germany, and EMU laboratories, UK, preferable accredited, can be used as sub-suppliers of analytical services. Analytical techniques utilized by Intertek laboratories are proven, accepted industry and scientific protocols. Individual laboratories are accredited to ISO 9000, ISO 17025, GLP and other standards depending upon the industries, markets and clients they service. Emu laboratory services are in accordance with quality systems including UKAS (ISO17025) and ISO9000:2000 accreditation. The laboratory services are underpinned by survey and project management teams, who work closely to achieve a comprehensive project, survey and monitoring service.

1.4.3 Presentation of the vendor

Alfa Laval and Wallenius Water AB have jointly developed and jointly own the PureBallast system through their joint venture company AlfaWall AB. AlfaWall AB is the vendor in this project.

1.4.3.2 Presentation of AlfaWall AB

AlfaWall is owned equally by Alfa Laval and Wallenius Water. AlfaWall is the legal product owner of PureBallast. The product is sold and distributed through the organization of Alfa Laval.

1.4.3.1 Presentation of Alfa Laval

Alfa Laval is a leading global provider of specialized products and engineered solutions. The equipment, systems and services are dedicated to helping customers to optimize the performance of their processes. Alfa Laval help customers to heat, cool, separate and transport products such as oil, water, chemicals, beverages, foodstuffs, starch and pharmaceuticals. The worldwide organization works closely with customers in almost 100 countries to help them stay ahead.

Alfa Laval also supplies a number of different products to the marine market such as fuel and lube oil separators, heat exchangers and fresh water generators. The company has in the last couple of years been widening its offering of solutions for environmental protection.

Alfa Laval is certified according to ISO 9000/2000

1.4.3.2 Presentation of Wallenius Water AB

Wallenius Water AB is a privately owned Swedish company located in Stockholm. The company is a wholly owned subsidiary of Wallenius Marine AB, a member of Soya Group. The company was founded in 1996 based on a new and patented technology. This technology is an Advanced Oxidation Technology (AOT) that has been found extremely potent in the treatment of water and removal of all kinds of microorganisms.

Apart from Ballast Water Treatment, Wallenius Water AB is present with its technology in other areas such as; Animal Husbandry, Horticulture, Cooling Systems, Industrial Process Water as well as potable water.

1.5 Responsibilities of all project participants

1.5.1 Shipboard installation, preparation of test tanks and clarification for shipboard testing

Shipboard installation, preparation of test tanks and clarification will be the vendors responsibility.

1.5.2 Installation, clarification and operation of the PureBallast treatment system

Installation and clarification and of the PureBallast treatment system will be executed by the vendor. Operation of the ballast water system, including treatment system, will be performed by the ships crew, assisted by vendor.

1.5.3 Biological water quality requirement

The biological requirement of the ballast water to be treated is a viable organism concentration at intake exceeding 10 times the values of Regulation D-2.1 and control tank viable organism concentration exceeding the values of Regulation D-2.1 on discharge. The vendor will be responsible of selecting seasons and locations to fulfil these requirements.

1.5.4 Sample collection and preservation

Tor Gunnar Jantsch and August Tobiesen at NIVA will be responsible for all sampling and preservation of samples connected to the chemical water quality and biological treatment performance during test cycles. Christian Vogelsang/Anne-Marie Bomo will step in for Jantsch if necessary, and Torsten Källqvist/Wenche Eikrem will step in for Tobiesen if necessary.

1.5.5 Clarifying test waters for discharge to recipient waters

The vendor will be responsible for the clarification of both treated and non-treated test waters before discharge to the local sea regions.

1.5.6 Laboratory chemical measurements

In general, the accredited NIVA laboratory will be responsible for chemical measurements: particulate organic carbon, total suspended solids and salinity. The accreditation warrant that the analyses are conducted according to internationally acknowledged quality systems (NS-EN ISO/IEC 17025) (Norwegian Accreditation 1993). In some cases, local laboratories, such as Intertek laboratories, Germany, EMU laboratories, UK, preferable accredited, can be used. Temperature will be measured "in situ".

1.5.7 Measurements of biological treatment factors

August Tobiesen will be responsible for the quantification of organisms >50 µm and ≥10-50 µm with stand-in Wenche Eikrem and Anne-Marie Bomo. Responsible for bacterial analyses, including, *E. coli*, intestinal *Enterococci*, *Vibrio* spp and *Vibrio cholerae* (serotypes O-1 and O-139) will be carried out by Tor Gunnar Jantsch with stand-in and assistance from Åse Bakketun, Anne-Marie Bomo and Christian Vogelsang. If necessary the analysis of, *E. coli* intestinal *Enterococci*, *Vibrio* spp and *Vibrio cholerae* (serotypes O-1 and O-139) can be transferred to an external, qualified laboratory. In some cases, local laboratories, preferable accredited, can be used.

1.5.8 Data handling and reporting of test results

The NIVA research manager Helge Liltved will be the main responsible for the reporting of test results to Alfa Laval/Wallenius Water AB. NIVA researchers Tobiesen, Jantsch, Bomo and Vogelsang will participate in data handling and reporting. All responsible personnel with stand-ins are given in Table 2.

1.5.9 Project management and coordination

Helge Liltved will be the coordinator and manager of the project.

1.5.10 Quality assurance of project

This QAPP will be approved by Det Norske Veritas (DNV). NIVA personnel involved with the internal quality assurance of the project will be:

Sampling	Helge Liltved
Laboratory chemical measurements	Håvard Hovind
Measurements of biological treatment factors	Torsten Källqvist
Data handling and reporting	Jarle Nygard

All NIVA staff involved in the practical project work will sign a letter stating that they have carefully read the QAPP and the non-disclosure agreement between AlfaWall (or Alfa Laval/Wallenius Water) AB and NIVA.

Table 2 Affiliation and responsibilities of all.

Responsibilities	Responsible personnel	Stand-in
Project management	Dr. Helge Liltved	
Ship responsible	Malin Westberg	Emil Eriksson
Preparation of test tanks on the vessel	Emil Eriksson	Peter Svensson, Per Borin
Installation and clarification of the PureBallast treatment system	Derek Appleby	Emil Eriksson
Operation of the PureBallast treatment system	Ships officers on M/V Aida	Emil Eriksson, Per Borin, Peter Svensson
Biological water quality requirement	Malin Westberg	Per Borin
Sample collection and preservation – chemical water quality	Dr. Tor Gunnar Jantsch	1. Dr. Christian Vogelsang 2. Dr. Anne-Marie Bomo
Sample collection and preservation – organisms ≥50 µm and ≥10-50 µm	Dr. August Tobiesen	1. Dr. Torsten Källqvist 2. Dr. Wenche Eikrem
Sample collection and preservation – bacteriology	Dr. Tor Gunnar Jantsch	1. Dr. Christian Vogelsang 2. Dr. Anne-Marie Bomo
Clarifying test waters for discharge to recipient waters	Malin Westberg	Per Borin
Laboratory chemical measurements	NIVA lab	Local laboratory
Measurements of biological treatment factors – organisms ≥50 µm and ≥10-50 µm	Dr. August Tobiesen	1. Dr. Anne-Marie Bomo 2. Wenche Eikrem
Measurements of biological treatment factors –*E. coli,* intestinal *Enterococci, Vibrio spp.,*and *Vibrio cholerae (*serotypes O1 and O139*)*	Dr. Tor Gunnar Jantsch	1. Dr. Anne-Marie Bomo 2. Dr. Christian Vogelsang 3. Techn. ass. Åse Bakketun 4. Local laboratory
Data handling and reporting of test results	Dr. Helge Liltved Participants: August Tobiesen, Tor Gunnar Jantsch, Anne-Marie Bomo, Christian Vogelsang	
Quality assurance of project – • QAPP • Sampling • Laboratory chemical measurements • Measurements of biological treatment factors • Data handling and reporting	 • Dr. Helge Liltved • Dr. Helge Liltved • M. Sc. Håvard Hovind • M.Sc. Torsten Källqvist • Jarle Nygard	

2. Experimental approach

2.1 Technology installation and shakedown procedures

2.1.1 General

The manual shall always be available during operation of the system (Alfa Laval, 2005). More information together with a system drawing is available in "Appendix H – Description and the PureBallast system and its components".

2.2 Technology start-up and stop procedures

2.2.1 System start-up procedures

See separate PureBallast operations manual.

2.2.2 System stop procedures

See separate PureBallast operations manual

2.3 Technology calibration checks

Engineering parameters that insure satisfactory operation of the PureBallast system are lamp operation, valve positions, flowrate, temperature and pressure levels are controlled by the control system and if no alarms are given, the system is operating satisfactory.
All measuring devices have been initially calibrated.

2.4 Test waters

Test waters will be pumped at the locations selected. The biological requirements stated in the IMO-Guildelines are given in Table 3.

Table 3 Required biological water quality in influent test water and in control and treated water on discharge.

Organism group	Influent/Uptake water	In control on discharge	In treated on discharge
≥50 μm min. dimension	>100 viable organisms per cubic metre	> 10 viable organisms per m^3	<10 viable organisms per m^3
≥10-50 μm min. dimension	>100 viable organisms per milliliter	> 10 viable organisms per ml	<10 viable organisms per ml
Escherichia coli			<250 cfu/100 ml
Intestinal *Enterococci*			<100 cfu/100 ml
Vibrio cholerae (Serotype O1 and O139			<1 cfu/100 ml

There are no requirements regarding chemical water quality. The source water (i.e. the influent water) should be analysed with respect to temperature, salinity, particulate organic carbon and total suspended solids.

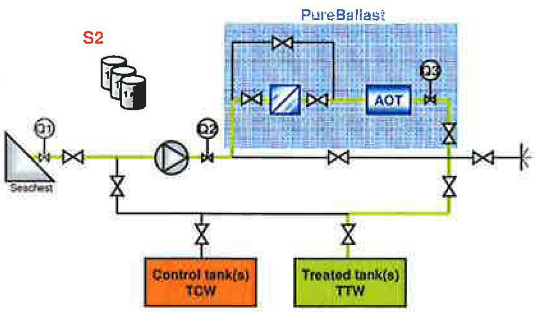
2.4.1 Assurance of fulfilment of biological water quality criteria

It is anticipated that the minimum concentration criteria can be difficult to fulfil for the different groups in surface water. Rapid tests based on microscopy will be performed to verify the presence of sufficient organisms according to point 2.2.2.5 in the Guideline: "Valid tests are indicated by uptake water, for both the control tank and ballast water to be treated, with viable organism concentration exceeding 10 times the values of Regulation D-2.1", as given in Table 3.

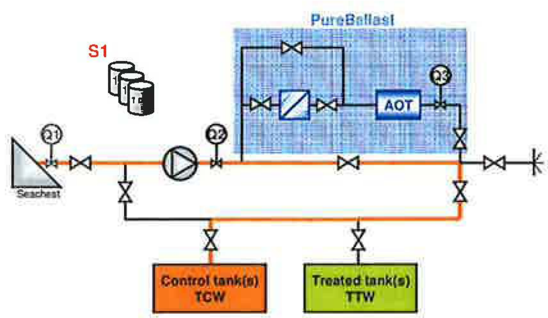
2.5 Running test cycles

The different water transfers between tanks via the PureBallast system during a test cycle including the control is shown in Figure 5. One test cycle involves consecutive treatment of test water during ballasting by the 50 µm filter unit and the Wallenius AOT Module of the PureBallast system (green lines) transferring the test water from the sea to a tank for treated water (TTW) (Figure 5, upper left). A control cycle (red lines) is run by ballasting seawater to a tank for control water (TCW) using the pump of the PureBallast system, but in by-pass of the treatment units (Figure 5, upper right). The deballasting of control water is also conducted by pumping only, without any treatment (Figure 5, lower left). A second treatment of the treated water is conducted by the Wallenius AOT unit during deballasting (Figure 5, lower right).

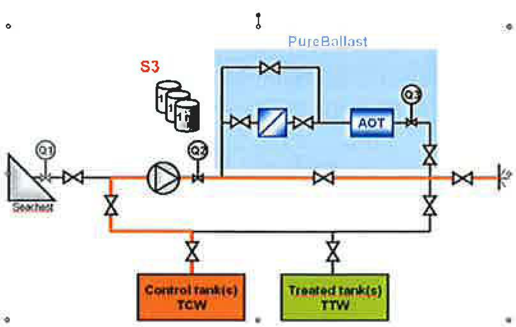
Ballast of treated water:



Ballast of control water:



Deballast of control water:



Deballast of treated water:

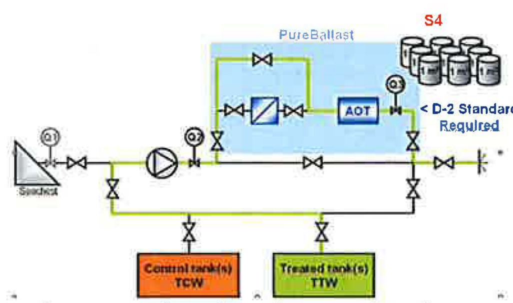


Figure 5 Illustration of ballast / deballast of test water for control tank and treated tank.

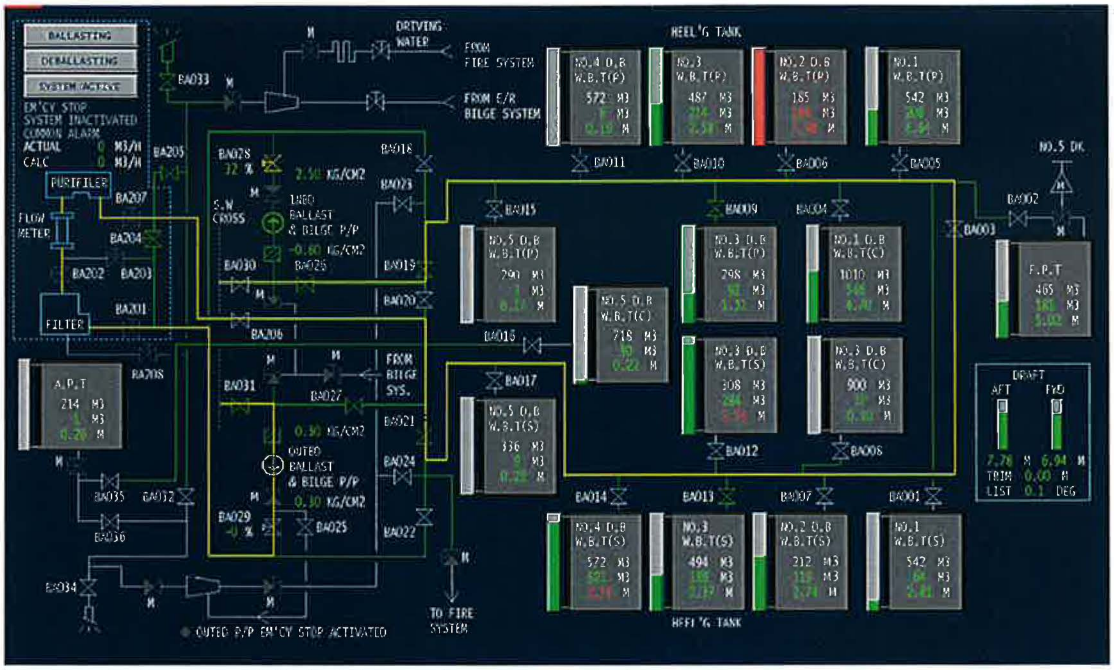


Figure 6 Cleaning of BW piping system prior to test

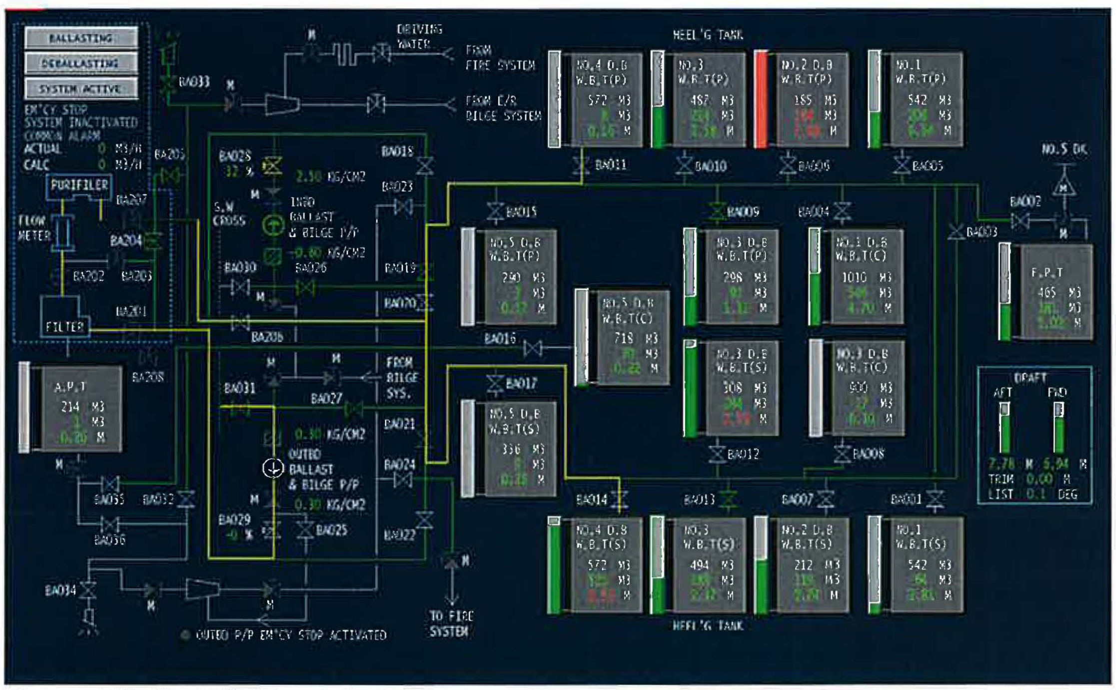


Figure 7 Ballast of of treated water

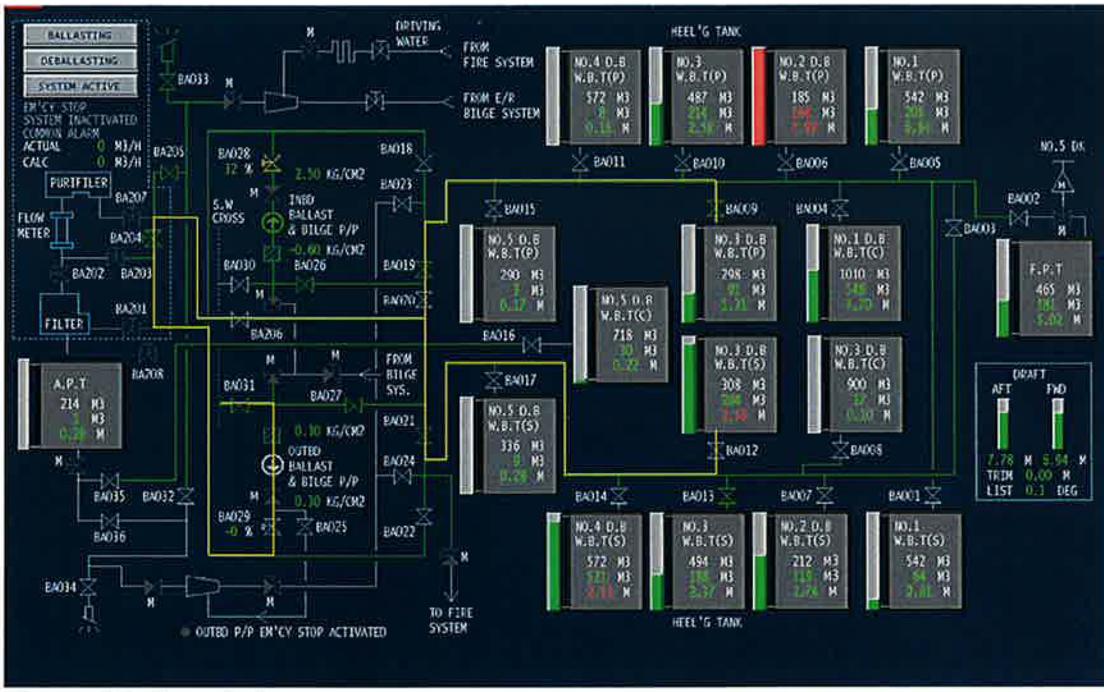


Figure 8 Ballast of of control water

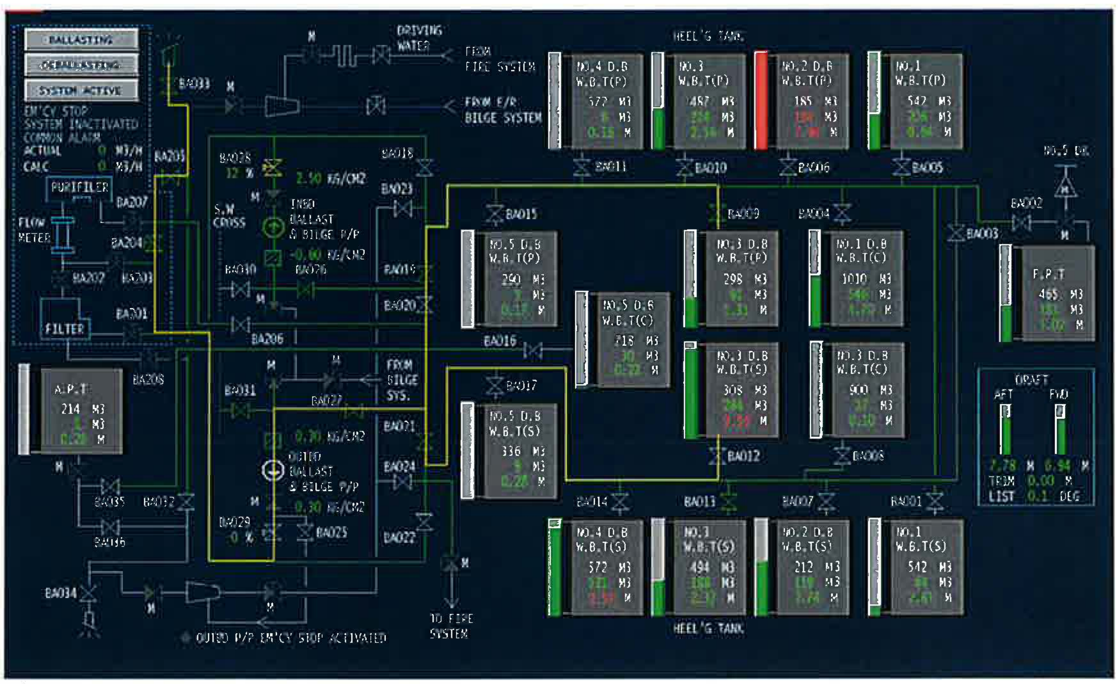


Figure 9 Deballast of of control water

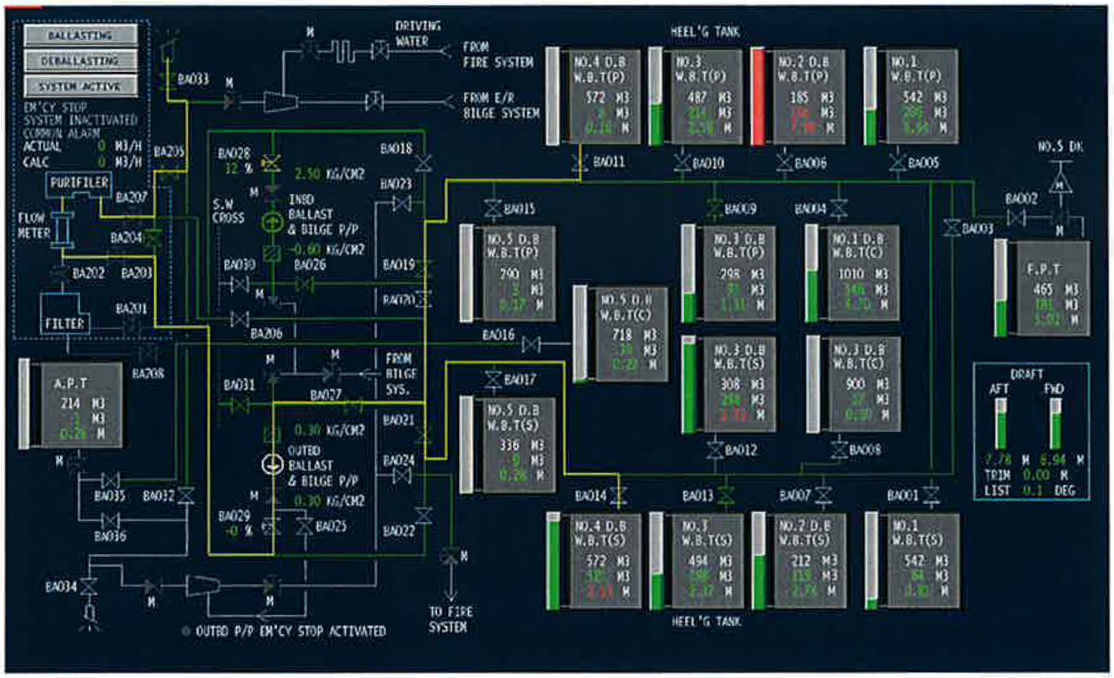


Figure 10 Deballast of of treated water

2.6 Sampling

The different sampling points at different water transfers (Table 4) between tanks when applicable via the PureBallast system during a test cycle including the control is shown in Figure 5. The influent water to the control tank is sampled (S1) from the sea chest (valve Q1) simultaneously with ballasting of the tank for control water (TCW) (Figure 5, upper right). The influent water to the treatment process is sampled (S2) from the sea chest (valve Q1) simultaneously with ballasting (and treatment of the water) of the tank for treated water (TTW) (Figure 5, upper left). The discharge control water is sampled (S3) from valve Q2 upon deballasting of the tank for control water (TCW) (Figure 5, lower left). The discharge treated water is sampled (S4) from valve Q3 simultaneously with deballasting (and deballast treatment) of the tank for treated water (TTW) (Figure 5, lower right). Valve Q2 and Q3 are equipped with sampling tubes described in Appendix K.

Table 4 Overview of sample collection.

Sample description	Abbreviation	Sampling point	Number of replicates
Influent water to control tank	S1	Seachest, valve Q1	3*
Influent water to treated tank	S2	Seachest, valve Q1	3*
Discharge control water	S3	Valve Q2	3**
Discharge treated water	S4	Valve Q3	9***

*Three replicate samples to be collected over the period of uptake (e.g. beginning, middle, end)
**Three replicate samples to be collected over the period of disharge (e.g. beginning, middle, end)
***Three replicate samples to be collected at each of three times during the period of discharge (e.g. 3 x beginning, middle, end)

Samples are withdrawn to document:

1) the source water chemical characteristics as defined by IMO,

2) that the water quality in the influent seawater is meeting the biological water quality criteria defined by IMO,
3) that the water quality in the discharge control water is meeting the biological water quality criteria defined by IMO,
4) that the water quality in the discharge treated water is meeting the biological water quality criteria defined by IMO,
5) the efficiency of the Alfa Laval PureBallast system in removing/inactivating target organisms in the ballast water upon discharge
6) the water quality in the control tank upon discharge .

Sampling numbers are indicated by S1-S4 in Figure 5 as described above.

Procedures for sampling and transfer are described in detail in section 3.

2.7 Sample analysis

Table 5 summarizes all types of measurements to be taken during the study.

Table 5 Parameters to be measured during the study; type of sampling and measurement location.

Parameter	Sample number	Type of sample	Location for analysis
Operational parameters for the PureBallast treatment system			
Chemical water quality measurements			
Temperature	S1, S2, S3, S4	*In situ*, continuous	M/V Aida
Salinity	S1, S2, S3, S4	Discrete grab	NIVA, local lab: Intertek laboratories, Germany, EMU laboratories, UK
Particulate organic carbon (POC)	S1, S2, S3, S4	Discrete grab	NIVA, local lab: Intertek laboratories, Germany, EMU laboratories, UK
Total suspended solids (TSS)	S1, S2, S3, S4	Discrete grab	NIVA, local lab: Intertek laboratories, Germany, EMU laboratories, UK
Biological treatment performance parameters			
Organisms > 50 µm	S1, S2, S3, S4	Discrete grab	M/V Aida
Organisms ≥10-50 µm	S1, S2, S3, S4	Discrete grab	M/V Aida and NIVA
E. coli	S1, S2, S3, S4	Discrete grab	M/V Aida NIVA, local laboratory
Intestinal *Enterococci*	S1, S2, S3, S4	Discrete grab	M/V Aida NIVA, local laboratory
Vibrio sp.	S1, S2, S3, S4	Discrete grab	M/V Aida NIVA, local laboratory
Vibrio cholerae (Serotype O1 and O139	S1, S2, S3, S4	Discrete grab	M/V Aida NIVA, local laboratory

2.8 Time schedule for the testing period

The time schedule of the testing period is shown in Table 6. The time schedule does not allocate time to unforeseen situations. Such situations may occur, and possible delays should be taken into account. According to the IMO-Guideline the test cycles should span a trial period of not less than six months including invalid and unsuccessful test cycles.

Table 6 Time schedule for testing.

Month 2007-2008	Sept	Okt	Nov	Dec	Jan	Feb	Mar	Apr	May
Tasks									
Technology installation	X								
Preparation planning	X			X			X		
Test cycle 1		X							
Test cycle 2					X				
Test cycle 3								X	
Quality assurance		X			X			X	
Data reduction/validation			X			X			X
Reporting									X

3. Sampling procedures

3.1 Representativeness of samples

Separate sampling lines from the valves to the sample points are used to assure that representative samples are drawn. Pipes are flushed with a volume corresponding to 1 times the piping volume (3 minutes) prior to sampling.

3.2 Sampling of test waters

Table 7 summarize which sampling equipment is used to collect samples for the individual parameters.

Table 7 Equipment and containers used for sampling and necessary and sampled volume for the individual parameters.

<table>
<tr><th>Parameter</th><th>Sampling equipment</th><th>Sample container</th><th>Collected volume S1, S2, S3, S4</th></tr>
<tr><td>Temperature</td><td>Temp. meter</td><td>-</td><td>-</td></tr>
<tr><td>Salinity</td><td>Probe</td><td>-</td><td>-</td></tr>
<tr><td>POC</td><td>Directly</td><td>Acid washed glass bottle</td><td>100 ml*</td></tr>
<tr><td>TSS</td><td>Directly</td><td>Clean plastic bottle</td><td>1000 ml*</td></tr>
<tr><td>Organisms ≥ 50 µm</td><td>Sieving**</td><td>Clean glass bottle</td><td>1 m^3</td></tr>
<tr><td>Organisms 10-50 µm</td><td>Directly</td><td>Clean glass bottle</td><td>1000 ml</td></tr>
<tr><td></td><td rowspan="5">Directly</td><td rowspan="5">Sterile plastic/glass bottle</td><td rowspan="5">1000 ml</td></tr>
<tr><td>E. Coli</td></tr>
<tr><td>Intestinal Enterococci</td></tr>
<tr><td>Vibrio sp.</td></tr>
<tr><td>Vibrio cholerae</td></tr>
</table>

* Might be combined depending on site of analysis.
** A 1 m^3 sample is concentrated to a volume of 40-100 ml through a plankton net with diagonal dimensions of 50 µm.

The procedures for collecting samples from sampling lines from the different sampling valves are as follows:

1) *Sampling of organisms ≥ 50 µm (S1, S2, S3 and S4).* A plankton net is placed in a plastic container and is used to collect a 1 m^3 sample. The sampled water is slowly sieved through a plankton net (50 µm diagonal dimensions) attached to a plastic cup, collecting the organisms in the cup. The volume of the sample is determined by measuring the volume passing out of the plastic container by a Siemens DN-50 flowmeter.
2) *Sampling of organisms 10-50 µm:* Organisms with a minimum diameter between 10 µm and 50 µm are sampled in 1000 ml bottles *(S1, S2, S3 and S4).*
3) *Sampling of bacteria (S1, S2, S3 and S4)*: Bacterial samples are sampled in 1000 ml sterile bottles.

3.3 Sample preservation

Preservation methods and expected storage/holding times before measurement are shown in Table 8.

Table 8 Preservation methods and expected storage/holding times before measurement (ISO/CD 5667-3, 2001).

Parameter	Preservation	Note	Maximum holding time	Expected storage time
Temperature	-	On board	-	0
Salinity	-	On board NIVA/local lab	-	0-7 days
Particulate organic carbon (POC)	Acidify with 1 ml 4 M H_2SO_4 per 100 ml(pH<2), 4°C	NIVA/local lab	7 days	0-5 days
Total suspended solids (TSS)	4°C	on board NIVA/local lab	24 hours	<24 hours
Organisms ≥ 50 µm	4°C	On board	6 hours	< 2 h
Organisms 10-50 µm	4°C and freezing	On board/NIVA lab	24 hours	< 24 h
E. Coli	4°C	On board/NIVA lab/local lab	24 hours	< 24 h
Intestinal *Enterococci*				
Vibrio sp.				
Vibrio cholerae				

3.4 Measures to avoid cross-contamination during test water transfer and sampling

Manual cleaning of the ballast tanks onboard M/V Aida is very difficult to manage due to the very limited access of the ballast tanks during ships operation. The manholes are only available when the ship carries no cargo.
Two of the ballast tanks intended to be used for testing PureBallast were visually inspected in June 2007. Only limited water remained after draining the tanks and very little sediment was present. Some viable species were identified on the tanks walls. It is however estimated that the contamination is limited and should not prevent the test program.

To avoid cross-contamination between consecutive test waters upon transfer in the ballastsystem, all pipes will be flushed to ensure that the entire water volume in the pipes has been replaced with treated water.before each sampling, see section 2.6. The volume of the ballast piping system is about 0,1 m^3 per meter pipe. The total volume should therefore be less than 20 m^3 (200 m pipe). It will take less than 3 minutes to flush the entire piping system. Plankton nets are dedicated to either treated or not treated sample water. Plankton nets will be rinsed between samples. All sample bottles will be thoroughly cleaned before use in sea water between each sampling.

3.5 Measures to secure sampling and analysis during rough weather conditions

Rough weather may cause a threat to safe sampling, sampling handling and analysis. Hence, sampling will only be conducted at times when secure handling of sampling containers at the sampling site, and upon transportation to the analysis facilities can be guaranteed. Samples will be stored in closed containers which are adequately secured in the analysis facilities, if rough weather develops between sampling and analysis. Petri dishes and dilution tubes will be stored in closed, secured containers during the analysis periods.

4. Testing and measurement protocols

An overview of all analytical measurements with instruments used, references and current uncertainty of the method applied is shown in Table 9 (chemical) and Table 10 (biological).

4.1 Biological measurements – initial quick tests

Rapid tests will be performed to verify the presence of sufficient organisms according to point 2.2.2.5 in the Guideline: "Valid tests are indicated by uptake water, for both the control tank and ballast water to be treated, with viable organism concentration exceeding 10 times the values of Regulation D-2.1. This would require that there is >100 organisms/ml of size >10-50 µm and >100 organisms/m^3 of >50 µm. Initial rapid tests are performed until the required organism concentration is achieved.

4.1.1 Rapid method of evaluation of density of 10-50 µm organisms

Filter 100 mL seawater sample from sea chest through 10 µm nitex net. Resuspend in 10 ml of seawater. Take out 100 µl and view at 40x magnification and count cells larger than 10 µm in minimum dimension. Target count is 100 cells in 100 µl of concentrated sample (target value >100 cells/ml).

4.1.2 Rapid method of evaluation of density of >50 µm organisms

Sieve 100 L seawater sample from sea chest through 40 µm Nitex screen. Resuspend in 10-50 ml. View sample in field microscope at 10x magnification counting organisms > 50 µm in minimum dimension. Sample should contain >10 organisms (target value > 100 organisms/m3).

4.2 In situ measurements

4.2.1 Temperature

Temperature is measured *in situ* using a calibrated thermometer. Temperature is reported in ^{o}C.

4.3 Discrete samples

4.3.1 Salinity

Salinity is measured on board and at the NIVA laboratory in Oslo using an accredited method (see Table 9).

4.3.2 Particulate organic carbon (POC)

POC is calculated as the difference between the level of total organic carbon in the sample, measured on the non-filtered sample, and the measured DOC level of the same sample.
DOC is measured by an accredited method based on Norwegian Standard NS-ISO 8245 (NIVA method G5-1) at NIVA after filtering the sample through a GF/F filter (0.7 µm). The sample is acidified with phosphoric acid and aerated with oxygen to remove inorganic carbon (NB! Removes also volatile organic carbon). The sample is injected in a quartz tube filled with a platinum catalyser at 680 oC. The organic carbon compounds are oxidized to CO2 which is quantified using an NDIR detector (Phoenix 8000 TOC-TC analyser with sample carousel STS 8000) with oxygen as carrier. Detection limit is 0.5 mg C/l.

4.3.3 Total suspended solids (TSS)

On board analysis is made of suspended solids by the Hach Method 8006; suspended solids, photometric method, 0-750 mg/L) measured by a handheld Hach spectrophotometer as the filtration, drying and weighing methods are considered unsuitable for use on-board due to the need for equipment. In the laboratory analysis a nucleopore capillary filter (0,4 µm) is dried at 40-50 ^{o}C for 2 hours and the tara is determined by weighing on a micro weight (Sartorius 4503 Micro) equipped with an ion source (Static Eliminator Bar Pu 210 Item no. LC 9793) removing static electricity. The sample is filtered through the filter, which again is dried at 40-50 °C for 2 hours before it is weighed on the micro weight. The TSS is represented by the weight increase. Lowest reported value: 0.1 mg/l. Ed. 2540 D pp 2-54: Total suspended dried at 103 – 105 °C. The method used is according to Standard Methods (APHA, 1995), but modified using nucleopore capillary filter in stead of glass fibre filter.

4.3.4 Organisms >50 µm

Organisms >50 µm are inspected in microscope at 10-40x magnification within 6 hours of sampling. Viable organisms are counted and identified based on motility and integrity according to OECD (1985): OECD Test Guideline for Testing of Chemicals 202, "*Daphnia* sp. acute immobilisation test and reproduction test". Viable organisms are identified to Phyla and to species level when possible. If organisms are found that are viable at the time of analysis the samples will be maintained for a period of time, either on board or in 1 liter bottles to be transported on shore, to investigate for how long these organisms will be viable.

4.3.5 Organisms ≥10-50 µm

CFDA-staining method

The viability of the micro-plankton (>10-50 µm) is determined by observing cells incubated with 5-carboxyfluorescein diacetate acetoxymethyl ester (CFDA-AM) according to Ganassin et al. (2000). A 10 ml sample is incubated for 1 hour with 4 µmol of CFDA-AM. The sample is fixed with formalin and filtered onto black polycarbonate filters (25 mm). The filter is mounted on a glass slide in paraffin oil and frozen. CFDA-AM is hydrolysed only in a living cell. CFDA-AM is a marker for cell membrane integrity and may be measured directly in cells. In principle, the non-fluorescent chemicals CFDA-AM is taken up in the cytosol, where it becomes hydrolysed into fluorescent end products. These end products are trapped inside the cellular compartment and may be observed in an epifluorescence microscope using excitation filter 485 nm and emission filter of 530 nm. In the epifluorescence microscope viable cells are observable as brightly yellow/green coloured cells, while non viable cells are pale green (heterotrophic cells) or pale green with red autofluorescence of the chloroplast (photoautotrophs). Numbers of viable and non viable cells are counted at a magnification of 300-480 times.

Dilution-culture method

The dilution-culture method is used as a complementary method for testing viability of organisms ≥10-50 µm. The method used is based on Throndsen (1978). Briefly, the dilution series is achieved by adding 1 ml sample to 9 ml of algal growth media (20% Z8 seawater media). After gentle but thoroughly mixing, 1 ml of this sample is further diluted with 9 ml of growth medium. In this way, a series of 10x dilution series are made. Number of dilution steps is set according to the expected cell density on the original sample. Five parallels are recommended to provide statistical significance of estimated number. The test-tubes are sealed/corked and incubated in a room or cabinet with suitable light and temperature conditions onboard ship until transportation in cooler chest to NIVA can be arranged where incubation can recontinued. After two to three weeks (depending on light and temperature conditions) the cultures are examined microscopically and the presence of each species in the tubes is noted. When the growth pattern (presence or absence) of each species through the culture series has been determined, the most probable number (MPN) per unit volume can be estimated from tables. Tables are given in Throndsen (1978).

4.3.6 *E. Coli*

Coliform bacteria are quantified according to Norwegian Standard NS 4788 (expand to e. coli here) at a temperature of 27±0.2 °C and an incubation period of 18-24 hours.

4.3.7 Intestinal *Enterococci*

Intestinal *Enterococci* are quantified according to Norwegian Standard NS-EN ISO 7899-2 or 7899-1 at a temperature of 44±0.2 °C and an incubation period of 44 hours.

4.3.8 *Vibrio spp.*

Vibrio spp. are quantified according to the method described by the American Public Health Association (APHA,1995). The total number of *Vibrio* sp., are determined by filtering of a 1-100 ml sample, the filter is placed on TCBS Cholera-medium agar plates (manufacturer: Oxoid), incubated at 35 °C, and the colonies are counted after 3 to 4 days.

4.3.9 *Vibrio cholerae* (Serotypes O1 and O139)

Vibrio cholerae identified above are confirmed for each colony from above by PCR and serotyping with specific antibodies for the outer antigens O1 and O139.

4.3.10 Sample transportation

For samples to be transported and analysed off-board the following applies.
Frozen 10-50 µm CFDA samples and the 10-50 µm dilution series are transported to NIVA as luggage with frozen cooling pack. If transportation back to NIVA is > 24 hours alternative handling may be required.
Samples for bacterial analysis are either transported off the ship to be analysed within the standard imeframe, or the samples will be filtered and incubated on-board. If the incubated plates must be taken from the ship prior to completion of the incubation, the incubation period on the ship will be extended for as long as possible, and the incubation will be continued as soon as possible on-shore.
Samples for chemical analysis are shipped in the same package but in a cooler 0-5 °C. Samples for bacterial analysis will be handled on board as fas as possible, but may be transferred on shore for continued incubation and analysis.

Table 9 Summary of all chemical measurements.

Parameter	Units	Instrument	NIVA method	Reference	Detection limit	Uncertainty			
						Control	#	Average	Std
***In situ* measurements**									
Temperature	°C		-	Instrument manual	0				
Discrete samples									
Salinity	PSU, ‰	Autosal model 8400A	A 3	UNESCO (1981)	0.005	Standard seawater IAPSO: 34,99252 PSU	9	34.9934	0.00105
POC	mg C/l		G5-3						
TSS	mg/l	Sartorius 4503 Micro weight	B4	Standard methods (1995)	0.1	Double analysis natural sample, TSM > 2 mg/l	32	0.9 % difference	11.1%

Table 10 Summary of all biological measurements.

Parameter	Units	Instrument	NIVA method	Reference	Detection limit
Organisms > 50 µm	#/m^3	Dissecting microscope 10-40x magnification	K 9	OECD Test Guideline (1985)	1/m^3
Organisms 10-50 µm	#/ml	Epifluorescence microscope (excitation filter 485 nm; emission filter 530 nm) at 300-480 times magnification	-	Ganassin *et al.* (2000)	1/ml
Organisms 10-50 µm	#/ml	Serial dilution technique	-	Throndsen (1978)	0,2/ml
E. Coli	cfu/100 ml	m-Endo Broth MF 274930 (Difco) after concentration by filtration	J2	Norwegian Standard NS 4788 (*expand to E. coli*)	1 cfu/100 ml
Intestinal *Enterococci*	cfu/100 ml	Specific agar, after concentration by filtration 44 °C, , verification on bile-esculinagar as coloured colonies	-	NS-EN ISO 7899-2 or 7899-1	1 cfu/100 ml
Vibrio spp.	cfu/100 ml	T.C.B.S. Cholera-medium Agar CM0333 (Oxoid) after concentration by filtration	-	APHA (1995), terminating the method after determining total count of *Vibrio* and prior to specific identification of *Vibrio cholerae*	1 cfu/100 ml?
Vibrio cholerae (serotypes O1 and O139)	cfu/100 ml	T.C.B.S. Cholera-medium Agar CM0333 (Oxoid) after concentration by filtration, PCR, Serotyping	-	APHA (1995), terminating the method after determining total count of *Vibrio* and prior to specific identification of *Vibrio cholerae*	1 cfu/100 ml

5. QA/QC checks

5.1 Data Quality Indicators (DQIs)

As part of the statistical analyses and assessment of the quality of data obtained for all performance measurements in the project, five data quality indicators (DQIs) will be used to interpret the degree of acceptability or utility of the data obtained in the project. These are representativeness, accuracy, precision, bias and comparability, and their protocols are described in the following sub sections.

5.1.1 Representativeness

The representativeness will be ensured by doing the following verification procedures:

1. Samples will be withdrawn only from water that has undergone treatment under normal operating conditions.
2. For the performance of the equipment, operating data will be measured at intervals throughout the period of testing ensuring a sufficient quantity of data to detect any change in operation. All alarms and events from the system are continuously logged by the control system. The flow is continuously, online, showed on the screen of the control system. The accumulated flow is calculated and stored in the control system.
3. All samples will be taken in triplicates.

5.1.2 Accuracy

Only accredited analytical procedures (according to NS-EN ISO/IEC 17025) will be used to describe critical parameters (e.g. imperative to verify that the required test conditions are fulfilled). The accuracy of these measurements is given in Table 9.

5.1.3 Precision

All samples will be taken in triplicates, and the standard deviation will be calculated. The acceptable analytical precision is ≤ 30 % for critical parameters.

Standard deviation, Std = $\sqrt{\frac{(X_i - X)^2}{n-1}}$ and % relative standard deviation = $\frac{100 \cdot Std}{X}$

where X_i = individual analytical result
X = the arithmetic mean of individual analytical results
n = number of measurements

5.1.4 Bias

To minimize possible bias results all samples will be taken in triplicates from the same volume of test water.

5.1.5 Comparability

One control test run will be conducted for each of the test waters as a reference and to identify any changes in critical parameters not caused by the equipment itself.

5.2 Emergency plan

This emergency plan shall be available at all times during testing. All persons in charge of technical equipment or similar connected to the issues listed below will be available at least by telephone during preparation of tests and during testing.

5.2.1 Power failure

The ship is equipped with an emergency power unit that will have start immediately when a power failure incidence takes place. The power unit will have the required capacity to supply lights and pumps of the test unit.

5.2.2 Pumping failure

If one of two the main pumps of the ballast water treatment system should fail, the other pump will be started. The risk of pump failure is very low due to the short pumping intervals during testing.

5.2.3 The right to veto between verifier and project group

The testing organization (NIVA) has the main responsible for the test procedure and quality assurance during testing. The captain of the ship has the main responsibility of safety on board and has the command in emergency situations. NIVA and AlfaWall personnel have the responsibility of collecting information about safety and safety procedures, and to follow these procedures during work on board. If a situation should arise were disagreement among the project group members regarding professional issues is evident, NIVA by the project manager on board has the right to decide what measures to be taken.

6. Data reporting, data reduction and data validation

6.1 Approach for data management and evaluation

Data will be recorded in standardized formats and in accordance with the following minimum requirements:

- Data are entered directly, promptly and legibly.
- Data are recorded legibly in ink. All original data records, as appropriate, a description of the data collected, the unit, the unique identification, the name of the person collecting the data and the date and time of data collection. All data will be scanned electronically and filed on a protected computer within 24 hours. All ink recorded data will be stored in a locker with restricted access.
- Any change to the original entry do not obscure the original entry, document the reason for the change and are initialled and dated by the person making the change.
- All deviations from the QAPP will be documented in writing and approved by the person in charge of the quality assurance of that particular part of the project. Documentation and communication include an assessment of the impact the deviation has on data quality.
- Data in electronic format will be included in commercially available programs for word processing, spreadsheet or database processing. Backup of computer databases will be performed on a daily basis.
- All results obtained with the PureBallast treatment system of Alf Laval/Wallenius Water AB will be handled with confidentiality in accordance with the established "Non-disclosure agreement"

All activities and collected data during testing of the PureBallast treatment system will be logged as summarized in Table 11. For each activity a specially designed log in paper and/or electronic format will be used, shown in Appendix A-H, and will be used for the respective quality assurance evaluation.

Table 11 Log protocols for all activities of the project.

Appendix	Description
A	Total project management
B	Collection and preservation of samples
C	Logging of *in situ* measurements
D	Evaluation form for organisms >50 µm
E	Evaluation form for organisms ≥10-50 µm
F	Evaluation form for heterotrophic bacteria, coliforms, Enterococcus group, intestinal *Enterococci*, *Vibrio cholerae* and *Vibrio cholerae* (serotypes O1 and O139).
G	Process Description
H	Description and the PureBallast system and its components

6.2 Reporting requirements

After the tests have been completed and the data have been evaluated, a report will be prepared and submitted to the Administration. The report will include information regarding test design, methods of analysis and the results of the analysis. Average values and standard deviations will be reported, but all the original data material will be available on request.

6.3 References

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Wet Chemical Oxidation IR-detection (EPA approved method no. 415.1 - STANDARD). Standard Methods 5310C ASTM D 4779 and D 4839.

Appendix A – Total project management

Cycle No. (of 3)	Date

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#	Description	Quality assurance	Peformed Yes	Name	Date	Sign.	Verifier	Avvik No.
Preparations – Day 0								
1	The PureBallast treatment system clarified for operation			Vendor				
2	The biological water quality requirement checked			ATO				
Sampling – Influent water control tank –S1								
3	Chemical samples collected and preserved							
	salinity ,POC, TSS			TGJ				
4	Chemical analyses performed							
	Temperature, salinity, SS			TGJ				
5	Biological samples collected							
	Organisms >50 µm			ATO				
	Organisms ≥10-50 µm			ATO				
	Microorganisms			TGJ				
Sampling – Influent water treatment tank –S2								
6	Chemical samples collected and preserved							
	salinity ,POC, TSS			TGJ				
7	Chemical analyses performed							
	Temperature, salinity, SS			TGJ				
8	Biological samples collected							
	Organisms >50 µm			ATO				
	Organisms ≥10-50 µm			ATO				
	Microorganisms			TGJ				
Sampling – Discharge water control tank –S3								
9	Chemical samples collected and preserved							
	salinity ,POC, TSS			TGJ				
10	Chemical analyses performed							
	Temperature, salinity, SS			TGJ				
11	Biological samples collected							
	Organisms >50 µm			ATO				
	Organisms ≥10-50 µm			ATO				
	Microorganisms			TGJ				
Sampling – Discharge water treated tank –S4								
12	Chemical samples collected and preserved							
	salinity ,POC, TSS			TGJ				
13	Chemical analyses performed							
	Temperature, salinity, SS			TGJ				
14	Biological samples collected							
	Organisms >50 µm			ATO				
	Organisms ≥10-50 µm			ATO				
	Microorganisms			TGJ				

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#	Description	Quality assurance	Peformed			Sign.	Verifier	Avvik No.
			Yes	Name	Date			
Analyses Influent water control tank –S1								
15	Chemical samples delivered for analysis			TGJ				
16	Chemical analyses performed			TGJ				
	POC							
	TSS							
17	Biological analyses performed							
	Organisms >50 µm			ATO				
	Organisms ≥10-50 µm			ATO				
	E. coli			TGJ				
	Intestinal Enterococci*s*			TGJ				
	Vibrio cholerae			TGJ				
Analyses Influent water treatment tank –S2								
18	Chemical samples delivered for analysis			TGJ				
19	Chemical analyses performed			TGJ				
	POC							
	TSS							
20	Biological analyses performed							
	Organisms >50 µm			ATO				
	Organisms ≥10-50 µm			ATO				
	E. coli			TGJ				
	Intestinal Enterococci*s*			TGJ				
	Vibrio cholerae			TGJ				
Analyses Discharge water control tank –S3								
21	Chemical samples delivered for analysis			TGJ				
22	Chemical analyses performed			TGJ				
	POC							
	TSS							
23	Biological analyses performed							
	Organisms >50 µm			ATO				
	Organisms ≥10-50 µm			ATO				
	Coliform bacteria			TGJ				
	Enterococcus group bacteria			TGJ				
	Vibrio cholerae			TGJ				
Analyses Discharge water treated tank –S4								
24	Chemical samples delivered for analysis			TGJ				
25	Chemical analyses performed			TGJ				
	POC							
	TSS							
26	Biological analyses performed							
	Organisms >50 µm			ATO				
	Organisms ≥10-50 µm			ATO				
	Coliform bacteria			TGJ				
	Enterococcus group bacteria			TGJ				
	Vibrio cholerae			TGJ				
Securing data								
27	All notes scanned and electronically saved			LIL				

Appendix B – Collection and preservation of samples

Sample Id.	Sample point
S1	Influent TCW
S2	Influent TTW
S3	Outlet TCW
S4	Outlet TTW

Purpose: Collection and preservation of samples		Water type			
Name:					
Date:					
Sample id.:					
		Volume	Filtration/ Preservation	Sign.	
Chemical samples					
Influent TCW (S1)					
POC					
TSS					
Influent TTW (S2)					
POC					
TSS					
Discharge TCW (S3)					
POC					
TSS					
Discharge TTW (S4)					
POC					
TSS					
Biological samples					
Influent TCW (S1)					
Organisms > 50 µm					
Organisms ≥10-50 µm					
Bacteria					
Influent TTW (S2)					
Organisms > 50 µm					
Organisms ≥10-50 µm					
Bacteria					
Discharge TCW (S3)					
Organisms > 50 µm					
Organisms ≥10-50 µm					
Bacteria					
Discharge TTW (S4)					
Organisms > 50 µm					
Organisms ≥10-50 µm					
Bacteria					

	Date, signature:
Operator	
Verifier	

Appendix C – Logging of in situ measurements

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Sample Id.	Sample point
S1	Influent TCW
S2	Influent TTW
S3	Outlet TCW
S4	Outlet TTW

Name:	
Date:	
Sample id.:	
Time of measurement	**Temperature °C**

	Date, signature:
Operator	
Verifier	

Appendix D – Evaluation form for organisms >50 µm

Page 1 of 1

Purpose: Preparation of biological water quality of test water			Water type	
Name:				
Date:				
Sample id.:				
		Observed species		**Phylum**
	Live			

	Date, signature:
Operator	
Verifier	

Appendix E – Evaluation form for organisms ≥10-50 µm

Page 1 of 3

Purpose: Documentation of live organisms ≥10-50 µm				Water type	
Name:	August Tobiesen			Salinity S:	
Date:				Brackish water:	
Sample id.:		1, 2, 3			
CFDA stained samples, inspected in epifluorescence microscope					
	Observed species			**Phylum**	
	Live/ ml				
1					
2		"		"	
3		"		"	
Average					
STDEV					

	Date, signature:
Operator	
Verifier	

Appendix E – Evaluation form for organisms ≥10-50 µm

Purpose: Documentation of live organisms ≥10-50 µm			Water type	
Name:	August Tobiesen		Salinity S:	
Date:			Brackish water:	
Sample id.:		1, 2, 3, 4, 5		
Dilution series				

	Observed growth					**Comments**
	Dilution step					
Sample	1	2	3	4	5	
1						
2						
3						
4						
5						
		95 % conf.limit				
	Total/ml	lower	upper			

Comments:

	Date, signature:
Operator	
Verifier	

Appendix E – Evaluation form for organisms ≥10-50 µm

Page 3 of 3

Purpose: Documentation of diversity ≥10-50 µm			Water type	
Name:	August Tobiesen		Salinity S:	
Date:			Brackish water:	
Sample id.:		Diversity		

Observed Species	Phylum

Appendix F – Evaluation form for E. coli, Intestinal *Enterococci and Vibrio spp., Vibrio cholerae* (serotype O-1 and O-139).

NIVA NORSK INSTITUTT FOR VANNFORSKNING	**Bakteriologiske analyseresultater**	J 2 = NS 4788 (Koli-37°) J 4 = NS 4792 (Koli-44°) J3 = NS 4791 (Kim-20)	J6 = NS 4793 (Strept)

Oppdragsnr.: Lab.kode:	Prøvetak. dato: Prøvetak. tid:	Lagringstemp	Mottatt NIVA dato: Analysert dato:	Rekvisisjonsnr:

METODE :											
Prøve nr.									Sign	Inkubator:	
Fortynningsgrad										Middeltemp	
Testporsjon, mL (V)										Temp. min.	
Fortynningsfaktor, (F)										Temp. max.	
Telte kolonier, antall (C)										**Tid inn:**	
Middelverdier										Tid ut:	
Antall pr. st. vol. (Vs)											
95 % konfidensintervall											
METODE NR:										Inkubator:	
Fortynningsgrad										Middeltemp	
Testporsjon, mL (V)										Temp. min.	
Fortynningsfaktor, (F)										Temp. max.	
Telte kolonier, antall (C)										Tid inn:	
Middelverdier										Tid ut:	
Antall pr. st. vol. (Vs)											
95 % konfidensintervall											
METODE NR:										Inkubator:	
Fortynningsgrad										Middeltemp	
Testporsjon, mL (V)										Temp. min.	
Fortynningsfaktor, (F)										Temp. max.	
Telte kolonier, antall (C)										Tid inn:	
Middelverdier										Tid ut:	
Antall pr. st. vol. (Vs)											
95 % konfidensintervall											

Forberedelse:	
Utførelse:	

g:\felles:\bakt:\baktarkx

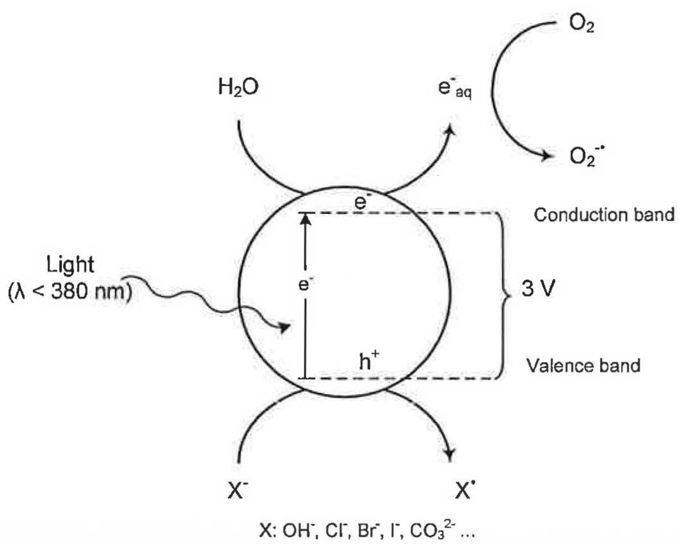
Appendix G – Process Description

This appendix describes the chemical features and discharge characteristics of the Wallenius-AOT process.

Chemical features

In sea-water, containing high concentrations of halide anions, charge-transfer absorptions will block all photons < 220 nm. (Platzman and Frank 1954, Treinin and Hayon 1975). This prevents any formation of ozone and related problems

Several metal-oxides exhibiting semi-conducting properties can be used as photo-catalysts in AOT-applications. Many of these applications are based on the specific properties of the anatase phase of titanium dioxide, TiO_2. In this material the absorption of a UV-photon of wavelengths below 387 nm can create a hole-electron pair of significant life-time (Kamat 1993, Legrini *et al.* 1993, Tada 2002, Fujishima *et al.* 2000, Diebold 2003).



If the hole $\oplus$ and the electron $\ominus$ can reach the surface of the photo-catalyst, they may cause oxidation (hole) and reduction (electron) transformations in the surrounding medium. In a fundamental study it has been shown that the electron enters the water medium becoming a solvated electron (Rothenberger *et al.* 1985), a state of well known chemistry with reaction times on the pico- to micro-second time scales (Baxendale and Busi 1982).

Typically, the solvated electron is trapped by oxygen in a diffusion controlled reaction forming superoxide, a free radical of low reactivity.

$$e^-_{aq} + O_2 \rightarrow O_2^{\bullet-}$$

Eventually, superoxide may disproportionate by formation of hydrogen peroxide:

$$2\,O_2^{\bullet-} + 2\,H^+ \longrightarrow H_2O_2 + O_2$$

Under the conditions prevailing in the AOT-treatment unit, hydrogen peroxide may undergo homolytic or reductive cleavage leading to formation of hydroxyl radicals, $^{\bullet}OH$:

$$H_2O_2 \rightsquigarrow 2\,^{\bullet}OH$$

$$e^-_{aq} + H_2O_2 \rightarrow HO^{\bullet} + OH^-$$

These reactions can be boosted by addition of hydrogen peroxide to the water entering the AOT-unit.

Hydroxyl radicals have superior oxidizing power and react exceedingly fast with most organic and inorganic constituents of sea water, *e.g.* with chloride ions (Baxendale and Busi 1982, Buxton *et al.* 1988, NDRL/NIST Solution Kinetics Database 2005).

When the positive hole, h+, reaches the surface it can oxidize adsorbed species on the particle surface. Thus, this reactivity is dependent on the adsorption of ions and other matter to the surface, goverened by the surface z-potential, which is pH-controlled (Tada 2002).

The hole, h^+, is a very strong one-electron oxidant that can react directly with adsorbed organic pollutants (including micro-organisms) or generate different oxidising radical species depending on the composition of adsorbed chemical species.

$$\oplus + X^- \longrightarrow X^\bullet$$

A typical sea-water contains about 0.5 M NaCl, 0.84 mM Br^- and 2.33 mM HCO_3^- (Horne 1969). Due to the high chloride concentration it is likely that the main adsorbed species on the photocatalyst surface is the chloride ion. Thus, a hole reaching the photo-catalyst surface probanly oxidizes a chloride ion to a chlorine atom:

$$\oplus + Cl^- \longrightarrow Cl^\cdot$$

This initial step is followed by a series of subsequent redox-reactions (Xiao-Ying 2004):

$$Cl^\cdot + Cl^- \rightleftharpoons Cl_2^{\cdot -}$$

$$Cl^\cdot + H_2O \rightleftharpoons H^+ + ClOH^{\cdot -} \rightleftharpoons Cl^- + {}^\cdot OH$$

Under prevailing conditions, i.e. near neutral pH and high concentration of chloride ions, predominant radicals acting in the system are dichloridyl ($Cl_2^{\cdot -}$), chlorbromidyl ($ClBr^{\cdot -}$), dibromidyl ($Br_2^{\cdot -}$) and carbonate (CO_3^-) radicals.

$$Cl_2^- + 2\,Br^- \rightarrow Br_2^- + 2\,Cl^-$$
$$Cl_2^{\cdot -} + CO_3^{2-} \rightarrow CO_3^{\cdot -} + 2\,Cl^-$$

These radicals are strong one-electron oxidants. Paradoxically, they are probably more bio-active than hydroxyl radicals because they may selectively attack essential proteins in the cell membranes of micro-organisms, a reaction which may lead to lysis and subsequent death (Engel *et al.* 1974, Ivanovskii and Mitrofanov 1978, Nogueira *et al.* 1998, Rijstenbil 2003, Zaafrane *et al.* 2004, Rijstenbil 2005).

UV-photons (□ about 260 nm) have also a direct lethal effect by destroying DNA., *e.g.* by pyrimidine lesions. Thus, free radicals and UV-photons kill micro-organisms synergistically. In addition, persistent pollutants, *e.g.* NDMA (N-nitrosodimethylamine), ATZ (atrazine) are oxidized and organo-halogen compounds like DDT and PCB can be reductively dechlorinated by trapping electrons on the photo-catalyst surface (Ahmed *et al.* 1999):

$$\ominus + R\text{-}Cl \longrightarrow R^\cdot + Cl^-$$

A problem related to ozone treatment of sea water is a significant formation of bromate, a suspected carcinogen. This reaction may proceed by way of an oxygen atom transfer mechanism. In the photo-catalytic process formation of bromate, BrO_3^-, is unlikely to occur, since this would require multiple one-electron oxidation steps. Moreover, the utilization of photocatalysts is one of the attractive methods for the removal of BrO_3^-. The reduction of BrO_3^- on a TiO_2 photocatalyst under UV irradiation is a well-known reaction, and bromate has even been used as an additive to improve the oxidation efficiency of TiO_2 photocatalysts (Oosawa and Gretzel 1988, Al-Ekabi *et al.* 1993, Mills *et al.* 1996, Lindner *et al.* 1997, Noguchi *et al.* 2003)

It should be pointed out that the radicals produced in the AOT-treatment unit are not different from those naturally formed by sun light in the surface layer of see-water. In this case, photo-cleavage of hydrogen peroxide and/or photo-oxidation of nitrate/nitrite ions may be the source of radical formation.

Discharge characteristics

The basic process operates without addition of chemicals using the synergistic effects of in situ produced free radicals and UV-photons to inactivate micro-organisms. In this system, the active substance is the anatase phase of TiO_2, which under UV-irradiation is classified as a biocide by the biocide directive of the European Union. *Per se*, TiO_2 is non-toxic as shown by its use in re-constructive surgery to obtain bone-compatible links for Ti-implants such as teeth and hip-joints (Pan *et al.* 1996, Diebold 2002). TiO_2 is also a widely used component in paints and cosmetic products. The action of reactive free radicals is of temporal nature, *i.e.* these species are so short-lived (micro-milli second time-scale) that they are not observable outside the AOT-treatment unit (Baxendale and Busi 1982, Buxton *et al.* 1988, NDRL/NIST Solution Kinetics Database 2005).

PureBallast has been tested for toxicity according to G9. PureBallast has subsequently been granted Final Approval.

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Appendix H – Description and the PureBallast system and its components

The PureBallast product is a complete system, composing of three main functions:

- Filter (50 µm)
  For removal of larger particles and organisms as well as to reduce the level of sediments in the ballast water tanks.
- Wallenius-AOT (Advanced Oxidation Technology) Unit
  A patented system product using naturally occurring photo-processes in the surface layer of sea-water. The Wallenius-AOT process acts on two levels: *in-situ* formation of reactive free radicals, and direct photo-effects. These two levels synergistically inactivate micro-organisms.
- Control and Auxiliary Equipment including sampling points
  The control system and transformer control the system and supply all items with power. The auxiliary equipment controls the flow and measure different alarm levels. The system also composes a CIP unit which is an automatic service device that cleans the quartz sleeves covering the UV-lamps after each Ballast or Deballast sequence. This operation is done to avoid scaling from seawater contaminants which would otherwise reduce the efficiency of the Wallenius-AOT Module.

Filter

S201-650 µm automatic back flushing filter

Wallenius-AOT unit

V201-19.1 Inlet valve for the Ballast water into the Wallenius-AOT Module. (DN 100)

V201-20.1 Outlet valve for the Ballast water from the Wallenius-AOT Module. (DN 100)

RV201-23.1 Relief valve to avoid if too high pressure in the Wallenius-AOT Unit would occur. The relief valve is set to 7 bars.

V321-2.1 The valve used for soft start of the Wallenius-AOT and circulation of CIP fluid to avoid pressure peaks in the Wallenius-AOT unit. (DN 40)

V321-3 Air relief valve for efficient drainage of the Wallenius-AOT Module.

V320-4.1 and V320-5.1 The valves should be open during the CIP process and during drainage of the Wallenius-AOT unit. The CIP process is circulating the CIP fluid from bottom to the top of the Wallenius-AOT module and then re-circulated to the CIP tank.

LS201-29 Level switch that detects the flow to get a signal when the AOT unit is full during soft start.

Control and Auxiliary Equipment including sampling points

Control System An overall control system that controls the process, log all data and give alarms.

Transformer Transformer that supplies all units with power.

Auxiliary Equipment

V201-3 Valve opened at ballasting for water flow through filter. Closed at deballasting for by-passing of the filter. Also used to open or close the total PureBallast system. (DN 250)

V201-9 Valve opened at ballasting for by-passing of the filter. Closed at deballasting. Also used to open or close the total PureBallast system. (DN 250)

V201-32 Outlet valve of treated ballast water. Opened both during ballasting and deballasting.

V201-8 Regulating valve to create a pressure difference over the filter to ensure efficient back-flushing, 2 bars needed. (DN 200)

IP201-7 Regulator for adjusting the valve V201-8.

PT201-27 Pressure Transmitter for controlling the pressure difference over the filter to have it as stable as possible around 2 bars.

V201-26 Valve to make it easier to exchange pressure transmitter (PT201-27).

FIT201-1 Flow meter that measures the amount of ballasted and deballasted water. This data is logged in the control system and adjusts the air regulation in the process.

PT201-16 Pressure Transmitter for adjusting the air regulation in the process.

V201-15 Valve to make it easier to exchange the pressure transmitter (PT201-16).

PI201-18 Pressure Gauge to be able to see the pressure direct in the process.

PD201-40 Protection for the Pressure Gauge to increase its life time. (Not standard in the PureBallast system)

V201-17 Valve to make facilitate exchange of the Pressure Gauge (PI201-18).

Sampling points

Q201.1 Sampling point for inlet water
Q201.2 Sampling point for outlet water
Q201.3 Sampling point for water after the filter and before Wallenius-AOT (not included in commercial system)

V201-2 Valve for sampling point Q201.1 (DN 50)

V201-43 Valve for sampling point Q201.3 (DN 50) (not included in commercial system)

V201-44 Valve for sampling point Q201.2 (DN 50)

V201-25 Injection and sampling point (Not standard in the PureBallast system)
V201-37 Injection and sampling point (Not standard in the PureBallast system)

CIP (Cleaning In Place)

T320-10 Tank for storage of CIP liquid between cleaning cycles and circling of CIP fluid.

P320-1Pump to circulate the CIP fluid.

LS320-7 Mini Squing to detect high flow in order to detect leakage of Ballast water into the CIP. (High Level Alarm)

LS320-8 Ultra sound detector to measure the level in the CIP tank. (Low Level Alarm) This is a safety component to protect the pump from overload and to detect potential leakages, correct opened and closed valves, that the tank has been filled with liquid etc. (Low Level Alarm)

V460-3 Manual valve for drainage of the CIP tank.

V320-2 Valve opened during the CIP process, draining of CIP

V321-1 Valve opened during the CIP process.

V460-2 Valve for drainage of Ballast water

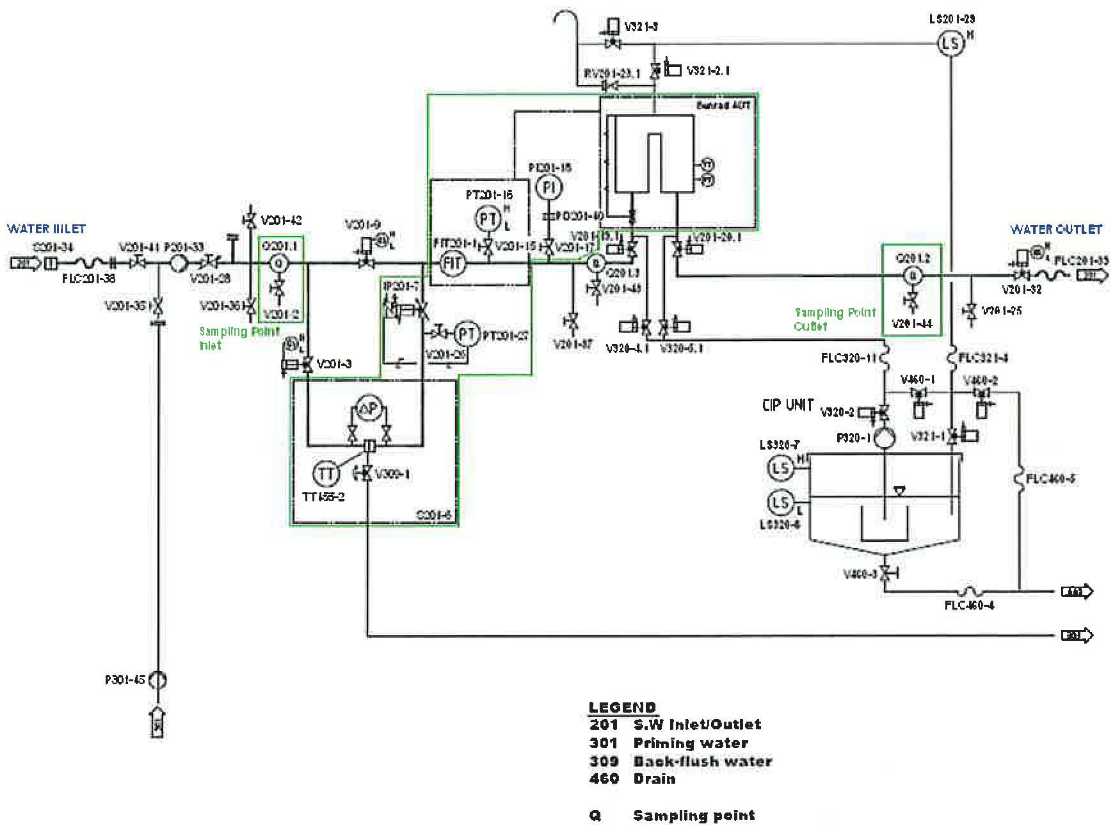
FLC320-11 Hose for CIP liquid from CIP tank to Wallenius-AOT.

FLC321-4 Hose for CIP liquid from Wallenius-AOT to CIP tank.

FLC460-4 Hose for drainage of the CIP tank.

FLC460-5 Hose for drainage of the Wallenius-AOT

Hoses are used to isolate the CIP tank from vibrations and to make the installation more flexible since the hoses easily can be extended or shortened.



Appendix I – IMO G8 requirements for shipboard tests

As outlined in the IMO requirements:

2.2 Shipboard tests

2.2.1 A shipboard test cycle includes:

.1 the uptake of ballast water of the ship;

.2 the storage of ballast water on the ship;

.3 treatment of the ballast water in accordance with paragraph 2.2.2.3 by the BWMS, except in control tanks; and

.4 the discharge of ballast water from the ship.

Success criteria for shipboard testing

2.2.2 In evaluating the performance of BWMS installation(s) on a ship or ships, the following information and results should be supplied to the satisfaction of the Administration:

[1] Such as ISO/IEC 17025.

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.1 Test plan to be provided prior to testing.

.2 Documentation that the BWMS is of a capacity within the range of the Treatment Rated Capacity for which it is intended.

.3 The amount of ballast water tested in the test cycle onboard should be consistent with the normal ballast operations of the ship and the BWMS should be operated at the Treatment Rated Capacity for which it is intended to be approved.

.4 Documentation of the results of three consecutive, valid test cycles showing discharge of treated ballast water in compliance with Regulation D-2.

.5 Valid tests are indicated by uptake water, for both the control tank and ballast water to be treated, with viable organism concentration exceeding 10 times the values of Regulation D-2.1 and control tank viable organism concentration exceeding the values of Regulation D-2.1 on discharge.

.6 Sampling regime:

.1 For the control tank:

.1 three replicate samples of influent water, collected over the period of uptake (e.g. beginning, middle, end).

.2 three replicate samples of discharge control water, collected over the period of discharge (e.g. beginning, middle, end).

.2 For treated ballast water:

.1 Three replicate samples of discharge treated water collected at each of three times during the period of discharge (e.g. 3 x beginning, 3 x middle, 3 x end).

.3 Sample sizes are:

.1 For the enumeration of organisms greater than or equal to 50 micrometres or more in minimum dimension, samples of at least one cubic metre should be collected. If samples are concentrated for enumeration the samples should be concentrated using a sieve no greater than 50 micrometres mesh in diagonal dimension.

.2 For the enumeration of organisms greater than or equal to 10 micrometres and less than 50 micrometres in minimum dimension, samples of at least one litre should be collected. If samples are concentrated for enumeration the samples should be concentrated using a sieve no greater than 10 micrometres mesh in diagonal dimension.

.3 For the evaluation of bacteria a sample of at least 500 millilitres should be taken from the influent and treated water.

.7 The test cycles including invalid and unsuccessful test cycles are to span a trial period of not less than six months.

.8 The applicant is requested to perform three consecutive test cycles that comply with Regulation D-2 and which are valid in accordance with paragraph 2.2.2.5. Any invalid test cycle does not affect the consecutive sequence.

.9 The source water for test cycles shall be characterized by measurement of salinity, temperature, particulate organic carbon and total suspended solids.

.10 For system operation throughout the trial period, the following information should also be provided:

.1 documentation of all ballast water operations including volumes and locations of uptake and discharge, and if heavy weather was encountered and where;

.2 the possible reasons for the occurrence of an unsuccessful test cycle, or a test cycle discharge failing the D-2 Standard should be investigated and reported to the Administration;

.3 documentation of scheduled maintenance performed on the system;

.4 documentation of unscheduled maintenance and repair performed on the system;

.5 documentation of engineering parameters monitored as appropriate to the specific system;

.6 documentation of functioning of the control and monitoring equipment.

2.2 Regulation D-2 stipulates that ships meeting the requirements of the Convention by meeting the ballast water performance standard must discharge:

.1 less than 10 viable organisms per cubic metre greater than or equal to 50 micrometres in minimum dimension;

.2 less than 10 viable organisms per millilitre less than 50 micrometres in minimum dimension and greater than or equal to 10 micrometres in minimum dimension; and

.3 less than the following concentrations of indicator microbes, as a human health standard:

.1 Toxicogenic *Vibrio cholerae* (serotypes O1 and O139) with less than 1 Colony Forming Unit (cfu) per 100 millilitres or less than 1 cfu per 1 gramme (wet weight) of zooplankton samples;

.2 *Escherichia coli* less than 250 cfu per 100 millilitres; and

.3 Intestinal *Enterococci* less than 100 cfu per 100 millilitres.

Summary of IMO requirements:

Testplan prior to testing

3 consecutive valid test cycles in accordance with D-2 for treated water discharged

Valid test: uptake 10x D-2.1, ctrl >D-2.1 (note 2.1, not D-2 here)

Sampling	CTRL	3 replicate influent (beg, mid, end), 3 samples
		3 replicate discharge (beg, mid, end), 3 samples
	Treated	3 replicates at three times discharge (beg, mid, end), 9 samples

Sample volume	>50	1 m3
	>10-50	1 liter bottles
	Bacteria	Sampling bottles (500 ml)
	Sal, temp, POC, TSS (source water, influent)	

Analysis different groups according to standard methods.

Regulation D-2:	<10/m3 >50 µm
	<10/ml >10-50 µm
	Vibrio cholerae (O1, O139)
	E. coli
	Intestinal *Enterococci*
Regulation D-2.1	<10/m3 >50 µm??
	<10/ml >10-50 µm??

Appendix J – List of Equipment required for sampling and analysis

Equipment for sampling	
Organisms >50 µm:	
40 µm Nitex screen for quick test	
1 m^3;to be filtered through plankton net (>50 µm cut off);	3 pieces of net necessary
Sampling bottles (100 ml) for filtered samples	18 + 2 pieces
Organisms >10-50 µm:	
Nitex net for quick test	1 piece
Sampling bottles (1000 ml) for grab samples from pipe	18 + 2 pieces
Bacteria:	
Sampling bottles (500 ml) for grab samples from pipe	18 + 2 pieces
Chemical measurements	
Sampling bottles (1000 ml) for grab samples from pipe	18 + 2 pieces
Equipment for analysis	
Organisms >50 µm:	
Stereoscope with counting chamber	1 piece
1 liter bottles	
Organisms >10-50 µm:	
Pipettes w/sterile tips	3 pieces
Staining procedure 5-carboxyfluorescein diacetate acetoxymethyl ester (CFDA-AM) Formalin Paraffin oil	
Glass slides	18 + 6 pieces
Filtration set	2 pieces
Freezing equipment/freezer on board	in situ
Bags for transportation of frozen samples	
Algal growth media (20% Z8 seawater media)	
Dilution glassware/Test tubes	
Bags for transportation of incubated samples	
Bacteria	
Incubation chambers	3 + 1 pieces
Incubation media (pre-poured in petri-dishes) *E. coli:* m-FC agar (or equivalent) Intestinal *Enterococci: Enterococcus* agar (or equivalent) *Vibrio cholerae* (O1 and O-139): TCBS agar (or equivalent)	 18 + 2 pieces 18 + 2 pieces 18 + 2 pieces
Sterile tweezers	4
Sterile dilution water	2 x 100 ml
Sterile filters	18 + 6 pieces
Sterile filtering equipment (single use)	18 + 2 pieces
Bags for transportation of Petri dishes (if necessary)	

Appendix K – Sampling tube